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Responses of Estuarine Phytoplankton Communities to Nitrogen Form and Mixing Using Microcosm Bioassays

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ABSTRACT: We examined the effects of different forms of nitrogen and mixed versus static conditions on the structure and function of natural Neuse River estuary phytoplankton communities incubated in 66-liter microcosms in March, May, August, and November 1999. Significant differences were found between effects of mixed versus static treatments in three of four experiments, but no differences were observed between effects of different forms of nitrogen. Mixed incubations resulted in higher contributions of diatoms to total community biomass (measured as chlorophyll *a*) than in static tanks in May. Significantly higher rates of carbon fixation were also observed, likely due to increased suspension of diatoms in surface (illuminated) layers of the tanks. In August, we found significantly higher abundances of cyanobacteria, total community biomass, and rates of carbon fixation in static tanks than in tanks that were mixed. In November, static incubations showed significantly higher abundances of cryptophytes resulting in higher total community biomass and rates of carbon fixation in static tanks than in mixed tanks. Nitrogen additions significantly increased total community biomass relative to controls in May and August, indicating that the communities were nitrogen-limited at these times. We conclude that while nitrogen additions may result in increases in phytoplankton biomass when nitrogen is limiting, phytoplankton community structure in the Neuse River Estuary may be determined more by the hydrodynamics of the system (mixing versus stratification) than by the form of nitrogen available for growth.

Introduction

The Neuse River estuary, North Carolina, currently shows symptoms of eutrophication that include recurring algal blooms, extensive bottom water hypoxia and anoxia, and fish kills (Paerl et al. 1990, 1998; Nixon 1996). High levels of nitrogen loading from the Neuse watershed and airshed have been implicated as the causative agents of eutrophication in this system (Paerl et al. 1990, 1995; Copeland et al. 1991; Pinckney et al. 1997, 1998). Productivity and growth of phytoplankton in the Neuse River estuary are at times nitrogen-limited (late spring, summer, early fall; see Hobbie and Smith 1975; Rudek et al. 1991; Paerl et al. 1995) making the estuary responsive to nitrogen inputs. While studies of eutrophication often examine the effects of nutrient inputs on bulk indicators such as primary productivity or total community biomass (usually measured as chlorophyll *a* [chl *a*]), our study takes a phytoplankton group-specific approach. Our underlying rationale is that the taxonomic composition and relative abundance of different algal groups in a phytoplankton community are fundamental determinants of aquatic ecosystem structure and function. Significant alterations in phytoplankton community composition could have major negative ecological and economic impacts on the entire estuarine ecosystem. Harmful and nuisance algal blooms, bottom water oxygen depletion, decreases in water quality, alterations of trophic structure, and the collapse of fisheries are all potential consequences of major shifts in community structure either at the algal group or species level.

Nitrogen inputs to the Neuse River estuary vary in chemical composition because they originate from diverse sources such as agricultural runoff or atmospheric deposition (Paerl et al. 1998; Pinckney et al. 1998). Intrinsic physiological differences in uptake capabilities between phytoplankton may result in species-specific or group-specific responses to different nitrogen forms and concentrations (Neilson and Larsson 1980; Collos 1989). Responses of phytoplankton to the form of available nitrogen may also be mediated by light availability. Light is a potentially limiting factor in turbid estuaries like the Neuse (e.g., Pennock 1985; Pennock and Sharp 1994), and there is some evidence that the Neuse is at times light-limited (Boyer et al. 1993). Nutrient uptake is directly or indirectly coupled to photosynthetic processes (Turpin 1991) because energy considerations determine, in part, what form of nitrogen is used by phytoplank-

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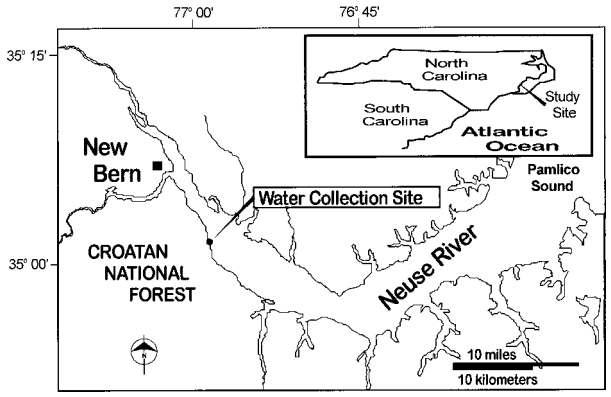

Fig. 1. Map of the Neuse River estuary in eastern North Carolina. The water collection site for the bioassays is indicated.

ton. Ammonium may be preferentially taken up over nitrate because less metabolic energy is required to assimilate the already-reduced ammonium form (Syrett 1981; Vincent 1992; but see Thompson et al. 1989). Phytoplankton responses to differing forms of nitrogen may be expressed as differential growth rates that will be ultimately manifested in the prevailing phytoplankton community structure and function; differential responses may play an important role in structuring natural phytoplankton communities and in mediating bloom dynamics (Stolte et al. 1994).

The overall goal of our study was to determine whether the form of nitrogen available for growth influences natural phytoplankton community structure and function in the Neuse River estuary. A second objective was to determine how mixing of the water column may affect phytoplankton community structure and function in this hydrodynamically variable system. Our overall hypothesis was that differing growth responses to various nitrogen compounds and mixing regimes (as a regulator of light exposure; see Cullen and Lewis 1988) would result in taxonomically-distinct and physiologically-distinct phytoplankton communities. We hypothesized that the energetic requirements for assimilation of nitrate will favor use of ammonium under conditions of reduced light availability (i.e., in static tanks).

Methods

We performed experimental manipulations of natural Neuse River phytoplankton communities that were collected from a mesohaline site in the Neuse River estuary (Fig. 1). Water was pumped from 1 m below the surface into a pre-cleaned (flushed with river water) 4,500-l trailer-mounted polyethylene tank using a non-destructive diaphragm pump and was transported to the Institute of Marine Sciences (IMS) in Morehead City, North Carolina. Previous work has shown that the location chosen for the water collection is representative of the phytoplankton community in the mesohaline section of the estuary (Pinckney et al. 1997, 1998). At IMS, the water was dispensed into 36 translucent 1 m tall fiberglass tanks, each with a 66 l capacity. The bottom two-thirds of each microcosm was wrapped with opaque material, while the top one-third of each microcosm was surrounded with two layers of neutral density screening (Fig. 2). Covering the microcosms prevented light from penetrating the tanks from the side and resulted in a vertical profile of irradiance similar to that measured in the Neuse River estuary, albeit over a compressed vertical scale (Fig. 2). Microcosms were amended with nutrients as outlined in Table 1. Nutrient additions were nitrate, ammonium, urea, nitrate + ammonium + urea in equimolar concentrations, and a combined treatment of nitrate + ammonium + phosphate. The combined treatment of nitrate + ammonium + phosphate allowed us to examine the potentially limiting or co-limiting effects of phosphate. The combined nitrogen treatments were included because phytoplankton communities are often exposed to nitrate, ammonium, and dissolved organic nitrogen in natural systems, and the possible interactive effects must be examined. We chose urea as a surrogate form of dissolved organic nitrogen (DON); we acknowledge that the composition and chemistry of DON compounds in the Neuse and other estuaries are still under study.

Microcosms were incubated outdoors in a large ($10 \times 10 \times 1.2$ m) concrete pond through which water from nearby Bogue Sound was circulated to maintain in situ temperatures, and were either left static or were gently mixed by bubbling slowly with air. Experiments were conducted in March, May, August, and November 1999. For each experiment, incubations were 6 d. As we were interested primarily in initial community responses and were wary of artifacts (container effects) that might occur with longer incubations, we focused on the first 3 d of each experiment. Nutrients were added to the tanks as concentrated solutions at 0800 on Days 0, 1, and 2 of each experiment so that the final concentration of added nutrients was as outlined in Table 1. There were six replicates of each nutrient addition treatment, three in mixed tanks and three in static tanks (Table 1). After the nutrient additions, each tank (including each static tank) was mixed thoroughly to distribute the nutrients throughout the tank. Microcosms were sampled immediately after nutrients were added and mixed; the samples taken reflect an integrated sample of the water column. Approximately 2.5 liters of water were taken from each microcosm

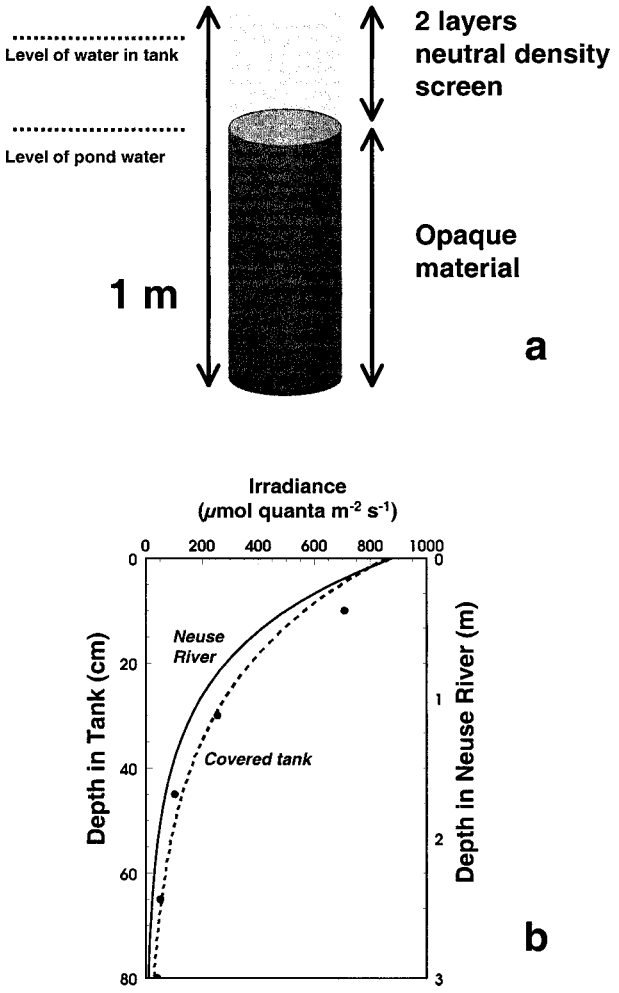

Fig. 2. a) Schematic representation of microcosms used in the present study. The top one-third of each tank was surrounded with 2 layers of neutral density screen. The bottom two-thirds was covered with an opaque material. Noted are the approximate levels of water on the outside of each tank when in the incubation pond and the level of sample water inside each tank. b) An example of a vertical profile of irradiance in microcosms used in the present study that contained Neuse River estuary water and a representative profile of irradiance in the Neuse River estuary. Note that covering the outside of the tanks results in the attenuation of light with depth in the tank similar to the attenuation of light in the estuary although over a compacted vertical scale.

each sampling day. Chemosystematic photosynthetic pigments, nutrient concentrations, and rates of carbon fixation were determined on Days 0, 1, and 3 of each experiment. Nutrient concentrations were determined on these days and on Day 6. Measurements of temperature and dissolved oxygen were done at the surface of the tanks on Days 0, 1, and 3 using a Hydrolab water quality profiler.

Photosynthetic pigments were analyzed by high performance liquid chromatography (HPLC) and

TABLE 1. Experimental design for microcosm bioassays. Nutrient additions were nitrate (N) alone (10 μM-N as KNO_3^-), ammonium (A) alone (10 μM-N as NH_4Cl), urea (10 μM-N) alone, nitrate + ammonium + urea (N + A + U, 3.3 μM-N + 3.3 μM-N + 3.3 μM-N), and nitrate + ammonium + phosphate (N + A + P, 5 μM-N + 5 μM-N + 3 μM-N). Phosphate was added as KH_2PO_4. Controls had no nutrients added. Nutrient additions were done for both mixed and static incubations for a total of 36 tanks. Results were analyzed statistically using a repeated measures analysis of variance with two fixed factors (nutrients, mixing) and Bonferroni post hoc analysis ($p < 0.05$).

Factors	Nutrient Addition	Concentration (μM-N or P)	Replicates of each treatment
Nutrients	Control	0	6
	Nitrate	10	6
	Ammonium	10	6
	Urea	10	6
	N + A + U	3.3 + 3.3 + 3.3	6
	N + A + P	5 + 5 + 3	6
Mixing	Mixed		18
	Static		18

were used to determine the composition and relative biomass of phytoplankton taxonomic groups (Millie et al. 1993; Jeffrey et al. 1997). The major phylogenetic groups of interest in the Neuse River were chlorophytes (with corresponding diagnostic pigments chl *b,* and lutein), cyanobacteria (zeaxanthin), diatoms (fucoxanthin), dinoflagellates (peridinin), and cryptomonads (alloxanthin) (Pinckney et al. 1997, 1998). Aliquots (0.2 to 0.8 l) were filtered under a gentle vacuum (< 50 kPa) onto 4.7 cm diameter glass fiber filters (Whatman GF/F) which were immediately frozen and stored at −80°C. Frozen filters were placed in 100% acetone (2 ml), sonicated, and extracted at −20°C for 12 to 20 h. Filtered extracts (200 μl) were injected into a Spectra-Physics HPLC equipped with a single monomeric (Rainin Microsorb-MV, 0.46 × 10 cm, 3 mm) and two polymeric (Vydac 201TP, 0.46 × 25 cm, 5 mm) reverse-phase C18 columns in series. A nonlinear binary gradient was used for pigment separations (Pinckney et al. 1996). Solvent A consisted of 80% methanol:20% ammonium acetate (0.5 M adjusted to pH 7.2), and solvent B was 80% methanol:20% acetone. Absorption spectra and chromatograms (440 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparing retention times and absorption spectra with pure crystalline standards, including chl *a, b,* β-carotene (Sigma Chemical Co.), fucoxanthin, and zeaxanthin (Hoffman LaRoche and Company). Other pigment identifications were based on extracts from phytoplankton cultures (Wright et al. 1991) and quantified using published extinction

coefficients (Mantoura and Llewellyn 1983; Rowan 1989; Jeffrey et al. 1997).

The contribution of each algal group to overall community composition in units of chl *a* was determined using CHEMTAX (CHEMical TAXonomy), a matrix factorization program (Mackey et al. 1996; Wright et al. 1996). This program uses steepest descent algorithms to determine the best fit based on an initial estimate of pigment ratios for algal classes. Both the absolute and relative contributions of algal groups to the total community biomass can be calculated. The absolute contribution of any algal group is the concentration of chl *a* (in $\mu g\ l^{-1}$) that is contributed by that group. Relative contributions are calculated as the proportion of total chl *a* that is accounted for by the group so that the sum of contributions from all groups equals 1. Initial pigment ratio files were taken from Mackey et al. (1996). Evaluations of the CHEMTAX method have shown it to be generally insensitive to values chosen for the initial pigment ratio matrix (Mackey et al. 1996; Schlüter et al. 2000). Full discussions, validation, and sensitivity analyses of CHEMTAX are provided in Mackey et al. (1996) and Wright et al. (1996).

Inorganic nitrogen and phosphate concentrations were measured to verify that nutrient additions in the bioassays were sufficient to increase nitrate, ammonium, and phosphate concentrations above those of natural water controls. Water samples (50–100 ml) were filtered through pre-combusted (500°C, 16 h) 25 mm Whatman GF/F filters before analyses. Nitrite + nitrate ($NO_2^- + NO_3^-$), ammonium (NH_4^+), and dissolved inorganic phosphate (PO_4^{-3}) were quantified in the filtered water with a Lachat AutoAnalyzer (Quickchem 8000) using standard protocols (Lachat Quickchem methods 31-107-04-1-C, 31-107-06-1-A, and 31-115-01-3-C, respectively). Nitrate, ammonium, and phosphate were the main nutrients of interest in our work. The addition of urea was done only as a preliminary experiment and therefore concentrations of this nutrient were not measured.

Rates of carbon fixation were determined by ^{14}C incorporation according to the method described by Parsons et al. (1984). One sample from each microcosm was transferred to a 125 ml polycarbonate bottle and was injected with $NaH^{14}CO_3$ (final concentration 185 to 260 kBq ml^{-1}). Each bottle was incubated in the tank from which the water sample was removed for 4 h centered at local noon. Bottles were weighted so that they were suspended at a depth in the tank that was approximately 50% of incident irradiance. The experimental design allowed triplicate determinations of carbon fixation for each nutrient and mixing treatment (one bottle from each tank) on each sampling day. One dark bottle per treatment was included and the value was subtracted from measurements in the light. After incubation, phytoplankton were filtered onto GF/F filters, air-dried, and fumed with concentrated HCl to remove unincorporated ^{14}C. Filters were then placed in vials containing scintillation cocktail (Ecolume, ICN, Inc.) and the counts per minute were enumerated with a Beckman Model LS5000TD liquid scintillation counter. Counts per minute were converted to disintegrations per minute using quench curves constructed from a calibrated ^{14}C-toluene standard. Dissolved inorganic carbon in all samples was determined by using a LiCor model LI 6252 CO_2 analyzer.

Responses of the phytoplankton community on Days 0 to 3 were analyzed using a repeated measures analysis of variance (ANOVA) (Neter et al. 1985; Scheiner and Gurevitch 1993) using SPSS 9.0 for Windows. Absolute concentrations of algal groups, total community biomass (as chl *a*), and carbon fixation data were analyzed using two fixed factors (nutrients, mixing) and were ln-transformed before analysis to satisfy the normality assumption. Relative abundance data were analyzed similarly, but were arcsine square root transformed. Homogeneity of error variances was checked using Cochran's Test, and because the assumption of homogeneity was satisfied a Bonferroni test ($\alpha = 0.05$) was used for post hoc comparisons of means.

Results

Water temperature in all microcosms varied between experiments following expected seasonal variations. Temperatures on Day 0 of each experiment were coldest in March (average temperature of all microcosms = 8.4 ± 0.1°C) with warmer temperatures in May, August, and November (16.9 ± 0.1, 26.1 ± 0.3, and 18.2 ± 0.2°C, respectively). Temperatures varied between Day 0 and Day 3 of each experiment by less than 2–4°C.

The initial composition and biomass of Neuse River phytoplankton communities collected at the mesohaline site also varied between experiments (Fig. 3). In March, the phytoplankton community was composed of chlorophytes, cryptophytes, cyanobacteria, and diatoms, contributing approximately 25.3%, 26.6%, 18.7%, and 29.4% of the total chl *a,* respectively. No dinoflagellates were observed. Total community biomass averaged 4.0 ± 0.7 (SD) μg chl *a* l^{-1}. In May, overall community biomass was higher than in March with a total chl *a* concentration of 12.8 ± 1.3 (SD) $\mu g\ l^{-1}$. Phytoplankton community composition in May was dominated by cryptophytes (51.2% of total community biomass), followed by diatoms (26%), chlorophy-

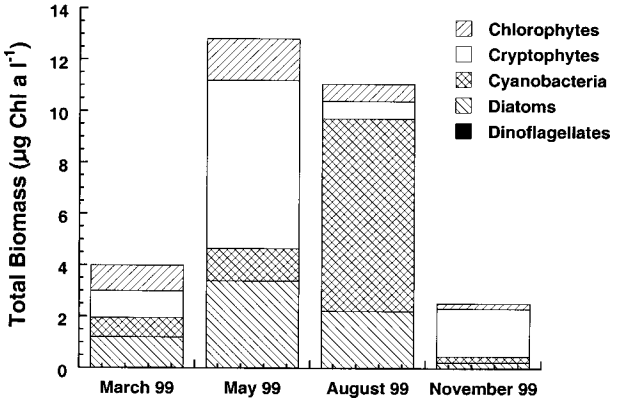

Fig. 3. Phytoplankton community composition at the study site on Day 0 of experiments conducted in March, May, August, and November 1999.

tes (12.6%), and cyanobacteria (10.1%). As in March, no dinoflagellates were detected. Cyanobacteria dominated the phytoplankton community at this site in August (68% of total chl *a*), while diatoms (20%), chlorophytes (6%), and cryptophytes (6%) made up the remainder. Total community biomass in August was 11.1 ± 1.7 (SD) µg chl $a\,l^{-1}$. Total biomass was lowest in the November experiment (2.5 ± 0.4 (SD) µg chl $a\,l^{-1}$). The November community was predominantly cryptophytes (73%), with contributions from the cyanobacteria (9.5%), diatoms (9%), and chlorophytes (8.4%). As was observed in all other experiments, no dinoflagellates were detected at this sampling location.

Responses to nutrient additions and mixing treatments varied between experiments. In March, no significant differences between controls and treatments were observed in phytoplankton community composition, total community biomass, or rates of carbon fixation (Fig. 4). In May, the absolute and relative abundances of cryptophytes were significantly higher in static incubations than in mixed tanks (Fig. 4). Mixing increased the absolute and relative contributions of diatoms in May resulting in higher rates of carbon fixation (Table 2 and Fig. 4), but there was no difference in overall community biomass. The addition of ammonium, nitrate + ammonium + urea, and nitrate + ammonium + phosphate resulted in significantly higher total community biomass (Fig. 4 and Table 2) through effects on the chlorophyte component of the community, although there was no significant change in the relative contribution of this group to phytoplankton community composition. Additions of different forms of nitrogen did not result in significant differences in community structure between treatment groups.

In August, the absolute and relative contributions of chlorophytes and diatoms to the phytoplankton community were significantly higher in mixed tanks than in static incubations (Fig. 4 and Table 2). Static incubations, however, resulted in higher absolute and relative abundances of cyanobacteria to the community, higher total community biomass, and higher rates of carbon fixation (Fig. 4 and Table 2) than mixing treatments. All nutrient treatments resulted in significantly higher absolute abundances of all algal groups present (chlorophytes, cryptophytes, cyanobacteria, and diatoms) and therefore in higher total community biomass and rates of carbon fixation (Fig. 4). There was a significant interaction observed between the static incubation and nutrient addition treatments (Table 2) for the absolute abundance of cyanobacteria. This is the only case where a significant interaction between the nutrient and mixing treatments was observed. The relative abundances of cyanobacteria and diatoms were significantly higher in all nutrient treatments than controls (Table 2 and Fig. 4).

We detected shifts in the total biomass and community composition of control tanks over the course of the August experiment. Total community biomass decreased from initial concentrations of 11.1 ± 1.7 (see Fig. 3) to 2.3 ± 0.2 (SD) µg chl *a* l^{-1} and 3.7 ± 0.2 (SD) µg chl $a\,l^{-1}$ in mixed and static control tanks, respectively. We also observed decreases in carbon fixation rates by nearly 4-fold between Day 0 and 3 in control tanks (Fig. 4).

There were no significant effects of nutrient additions on total community biomass or on community composition in November 1999. There were significant effects of mixed versus static treatments. Mixing increased the absolute and relative abundance of cyanobacteria and diatoms to the community (Table 2 and Fig 4). Cryptophytes were significantly higher in static incubations than in mixed tanks, and the relatively high proportion of cryptophytes in the community resulted in higher total community biomass and rates of carbon fixation in the static incubations than in mixed tanks (Fig. 4 and Table 2).

Initial concentrations of dissolved inorganic nutrients varied widely between experiments and further additions of nitrate, ammonium, or phosphate to the microcosm tanks resulted in varying concentrations of each of these three nutrients. Representative data from the May and August experiments are shown in Fig. 5. Initial concentrations of nitrogen (particularly nitrate) were high in May as was observed in March and November. Both nitrate and ammonium were initially extremely low (< 1 µM-N) in August (Fig. 5). Concentrations of all nutrients declined between Days

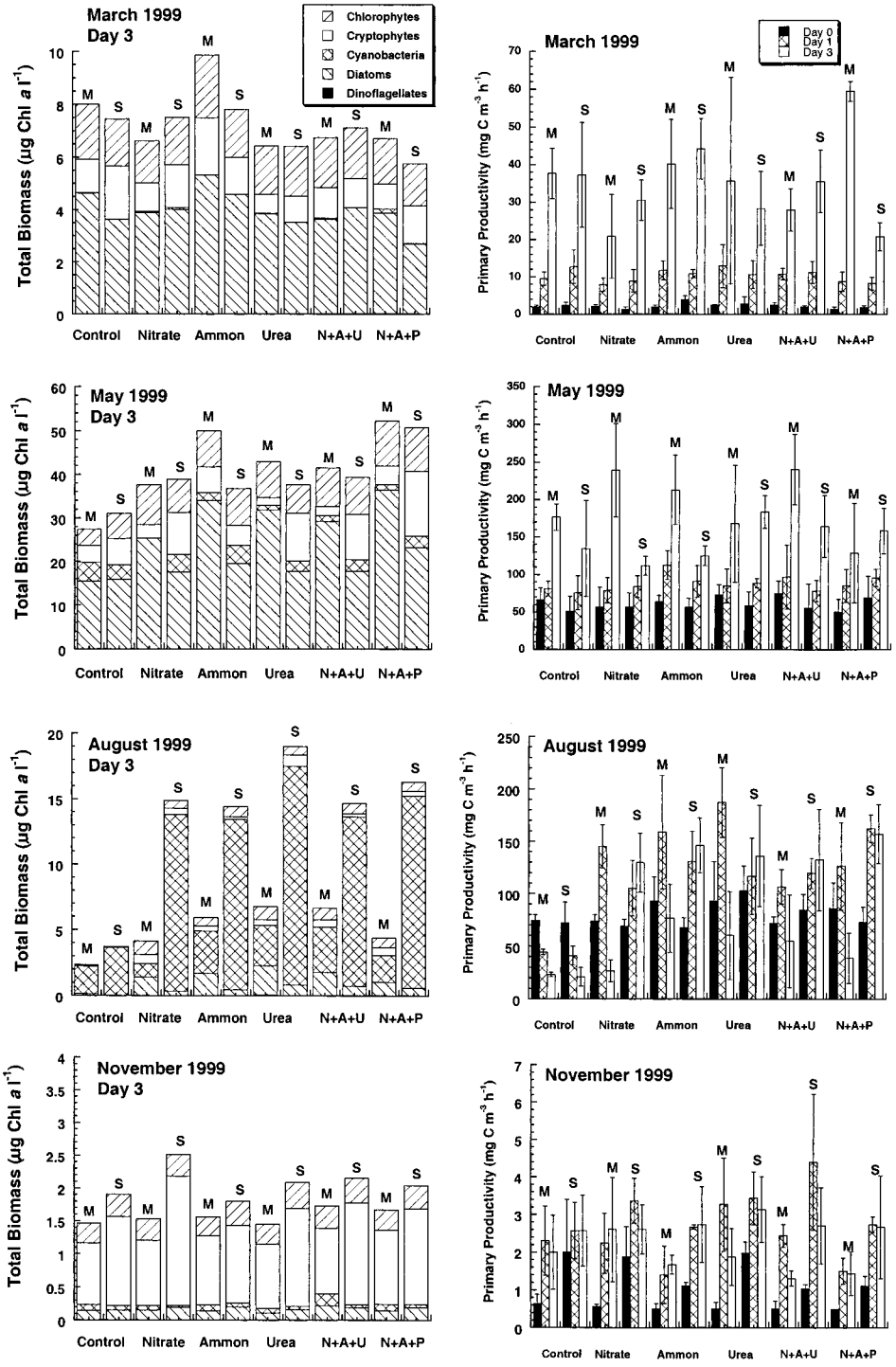

Fig. 4. Phytoplankton community composition (Day 3 only) and rates of carbon fixation from experiments conducted in March, May, August, and November 1999. Microcosms were amended with nitrate (N), ammonium (ammon; A), urea (U), N + A + U in equimolar concentrations, or N + A + phosphate (P) as detailed in Table 1. M and S refer to mixed and static tanks, respectively. Error bars indicate standard deviation of the mean.

TABLE 2. Results of 2 factor repeated measures ANOVA for each experiment in 1999. The symbol C denotes the control treatment, N refers to the nitrate addition treatment, A to the ammonium addition treatment, U to the urea addition, NAU to nitrate + ammonium + urea, and NAP to nitrate + ammonium + phosphate additions. Absolute and Relative refer to the absolute concentration of the algal group and the relative abundance of the group in the community, respectively. Non-significant differences are indicated by − and, where differences were detected, the results of mean comparisons are presented ($p < 0.05$). * indicates a significant interaction between mixing and nutrient treatments.

Variable	Factors	Abundance Measure	March	May	August	November
	Mixing		—	—	static > mixed	static > mixed
Primary Production	Nutrient		—	—	all additions > C	—
	Mixing		—	mixed > static	static > mixed	static > mixed
Total Biomass	Nutrient		—	A, NAU, NAP > C	all additions > C	—
		Relative	—	mixed > static	mixed > static	mixed > static
	Mixing	Absolute	—	mixed > static	mixed > static	mixed > static
		Relative	—	—	A, U, NAU > C	—
Diatoms	Nutrient	Absolute	—	—	all additions > C	—
		Relative	—	static > mixed	—	static > mixed
	Mixing	Absolute	—	static > mixed	—	static > mixed
		Relative	—	—	N > C	—
Cryptophytes	Nutrient	Absolute	—	—	N, A, NAU, NAP > C	—
		Relative	—	—	mixed > static	—
	Mixing	Absolute	—	—	mixed > static	—
		Relative	—	—	—	—
Chlorophytes	Nutrient	Absolute	—	N, A, NAU, NAP > C	all additions > C	—
		Relative	—	—	static > mixed	mixed > static
	Mixing	Absolute	—	—	static > mixed*	mixed > static
		Relative	—	—	all additions > C	—
Cyanobacteria	Nutrient	Absolute	—	—	all additions > C*	—

3 and 6 of each experiment because no further nutrient additions were made after Day 2.

Discussion

A variety of biological, chemical, and physical processes regulate phytoplankton community structure and function in aquatic ecosystems. Abiotic factors, such as nutrients, light, temperature, salinity, and vertical mixing, and biotic factors, like inter-specific competition and selective grazing, have all been implicated as major structuring forces. The composition of natural phytoplankton communities reflects the integrated effects of these known (and unknown) selective pressures. An understanding of the integrated effects is fundamental to the development of predictive ecological models and to the formulation of effective coastal nutrient management strategies (Cloern 1996; Roelke et al. 1997). This can only be achieved through manipulations of individual structuring factors under controlled experimental conditions, e.g., in microcosms. The high degree of variability that we observed in initial community composition during our experiments reflects the integrated effects of various forcing factors. This variability is consistent with the high degree of temporal and spatial variability in phytoplankton community composition in the Neuse River estuary characterized by previous work (Pinckney et al. 1997, 1998; Paerl et al. 1998). The differences in initial phytoplankton community composition that we observed between seasons in this study makes it difficult to separate differential growth responses due to initial community structure from seasonal differences, at least with this experimental design.

Responses to Nutrient Additions

Previous work has hypothesized that different forms of nitrogen may result in taxonomically-distinct phytoplankton communities due to inherent differences in uptake capabilities and preferences (Eppley et al. 1969; Carpenter and Guillard 1971; Dortch 1990). We found no significant differences between the effects of different forms of nitrogen on phytoplankton community structure or function. Our work agrees with a study by Burford and Pearson (1998) that also showed that the form of available nitrogen had no effect on phytoplankton community composition in aquaculture ponds in Australia. We acknowledge that it was difficult to separate completely the effects of different nitrogen forms in some of our experiments, because high concentrations of ambient nitrate in winter months confounded our attempt to alter the ratio of available nitrate to other nitrogenous nutrients. Variations in the experimental design are necessary to resolve completely the issue of nitrogen form under these conditions, but there was still no clear evidence of significant differences between different forms of nitrogen even when ambient nitrogen was low or undetectable. The isolation of water in microcosms away from underlying sedi-

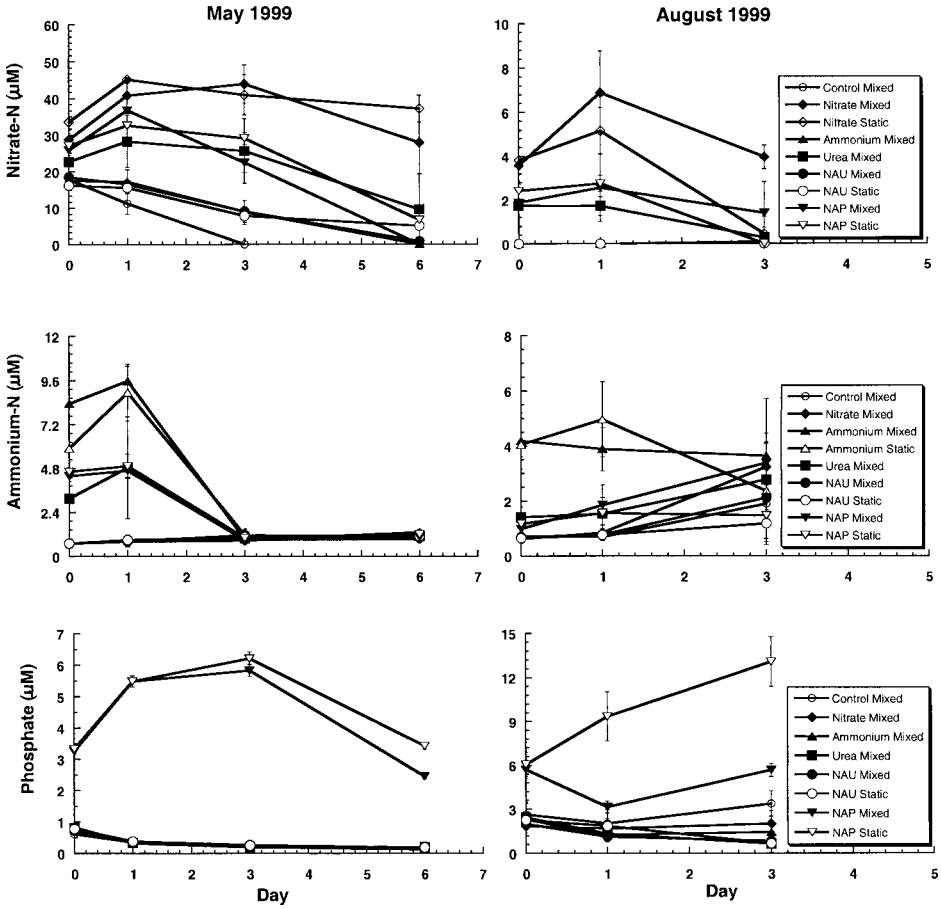

Fig. 5. Dissolved inorganic nitrogen (nitrate-N and ammonium-N) and dissolved inorganic phosphate concentrations from representative nutrient treatments in May and August 1999. Error bars indicate standard deviation of the mean. An impending hurricane prevented sampling beyond Day 3 of the August experiment.

ments also prevented us from examining any effects of sediment-derived nitrogen fluxes to this system.

Neuse River estuary phytoplankton responded to nitrogen additions in all forms and concentrations only when ambient nitrogen concentrations were initially low. High ambient concentrations of nitrate in March and November are the likely explanation as to why nitrogen additions did not significantly affect rates of carbon fixation, overall community biomass, or phytoplankton community structure; phytoplankton communities during these experiments were already nitrogen-replete. In May, initial concentrations of nitrate were fairly high (> 10 μM), but high initial biomass meant that phytoplankton quickly depleted the tanks of both nitrate and ammonium. In August, initial ambient nitrogen was extremely low and nitrogen was taken up readily by phytoplankton in a classic nitrogen-limited response. Previous work has shown that phytoplankton in the Neuse River estuary are nitrogen-limited during the springtime, summertime, and into the fall (Rudek et al. 1991; Paerl et al. 1995) being especially pronounced in the summer months. Our experiments support these observations.

Manipulations performed in March 1999 elicited no response of the phytoplankton community to nutrient additions. While the lack of response is likely due in part to the high ambient nitrogen concentrations, it is important to note that cells of the usual winter (February/March) bloom-forming dinoflagellate, *Heterocapsa triquetra,* were not present at this time. Short day length, relatively low sun angle, and cold temperatures in March may also have played a role.

Results of the May experiment were particularly interesting in that the community seemed to show a preference for ammonium over nitrate, or possibly the inhibition of nitrate uptake in the presence of ammonium (see, e.g., Dortch 1990). By Day 3 of the experiment, there was still approxi-

mately 10 μM nitrate in the ammonium addition tanks but virtually all of the ammonium had disappeared. Any preferences for uptake that may have existed, however, were not translated into significant differences in phytoplankton community structure and function. Possible preferences for ammonium over nitrate also do not seem to be related to light availability (and thus energetic requirements) as the trend was observed under both mixed and static conditions. It is possible that this preference is related to the taxonomic composition of the community.

Possible Effects of Zooplankton Grazing

In the August experiment, total community biomass was initially 11 μg l^{-1}. By Day 3 of the experiment, biomass in control tanks had decreased to between 2–4 μg l^{-1}, indicating that there was substantial grazing by zooplankton occurring in the tanks. Effects of grazing are also seen in measurements of carbon fixation in August (Fig. 4). Carbon fixation in controls and mixed tanks declined by Day 3; growth of cyanobacteria in the static tanks was sufficiently rapid to allow accumulation of biomass over losses due to grazing. Calculations of primary productivity normalized to total chl *a* show that assimilation numbers did not decline over the course of the experiment. This indicates that the community was growing well and supports the notion of top-down control of phytoplankton biomass. Previous work in the Neuse River estuary has shown that zooplankton community grazing rates are generally lowest in winter and highest in spring and late summer (range 0.1 to 310 ml l^{-1} h^{-1}) and may account for between 38–45% of daily phytoplankton production in this estuary (Mallin and Paerl 1994). These results illustrate clearly that the possible top-down effects of grazing on phytoplankton community structure and function, including the possibility of group-specific grazing, should also be considered.

Response to Mixed versus Static Treatments

The most noticeable response of Neuse River phytoplankton communities was to the mixed versus static treatments. Carbon fixation was significantly higher in mixed tanks than in static tanks in experiments conducted in May, indicating that light availability may limit or co-limit primary productivity and growth. In the Neuse River estuary, turbidity is high and light is rapidly attenuated with depth. The diffuse attenuation coefficient (K_d, m^{-1}), a factor that describes the extinction of light with depth (Kirk 1994), ranges from less than 1 to greater than 4 m^{-1}, averaging 2.2 ± 0.79 (SD) m^{-1} (Pinckney unpublished data). Because of high turbidity, primary productivity may be reduced or light-limited even at times when there is an ample supply of nutrients. Light-limitation of phytoplankton production has been examined in other estuaries (Wofsy 1983; Cole and Cloern 1984; Pennock 1985; Harding et al. 1986; Cloern 1987; Pennock and Sharp 1994) and there is supporting evidence from the field that Neuse River phytoplankton populations may be at times light-limited (Boyer et al. 1993). Direct extrapolation of results of our work in 1 m tall microcosms directly to the Neuse River must be done with caution. We have not quantified the rate of vertical mixing in our tanks, and do not know how our mixing rates compares to those in the river. We used gentle bubbling simply as a mechanism to keep the phytoplankton in suspension and to increase the amount of light available to the phytoplankton community. We acknowledge the importance of assessing rates of turbulent mixing in the microcosms (Sanford 1997) and this is an area that must be addressed in future research. Future studies should also include an examination of the potentially detrimental effects of mixing on zooplankton abundance in our system (Petersen et al. 1998) and the possible effects of the ratio of radius to depth of microcosms which has been shown to be an important consideration (Petersen et al. 1997).

The diatom and chlorophyte components of the Neuse phytoplankton community seemed to be particularly responsive to the mixing treatment. In the August and November experiments, both the absolute and relative contributions of the diatoms to the phytoplankton community were significantly higher in mixed tanks than in static tanks. We considered the possibility that the increased abundance of diatoms in mixed tanks was an artifact of sampling. It is possible that the relatively heavy diatoms sink to the bottom of static tanks and thus were not effectively sampled. However, before sampling, all tanks (mixed and static) were thoroughly mixed and an integrated sample was taken, minimizing the risk of such a sampling artifact. Diatoms often flourish in mixed systems (Margalef 1978). This is sometimes considered to be due to increased nutrient supply due to turbulent diffusion and/or advection, but nutrient concentrations in our tanks were comparable. Mixing of the tanks should result in increased suspension of diatoms and chlorophytes and therefore increased residence time in the more highly illuminated upper layers of the tanks. This is the more likely explanation of the response of these organisms to the mixing treatment. Our observations are consistent with the data of Pinckney et al. (1999) that showed that mixed conditions were conducive to increased rates of carbon fixation and abundance of chlo-

rophytes and diatoms from the same location in the Neuse River estuary.

While diatoms and chlorophytes seemed to prefer mixed conditions, cyanobacteria and cryptophytes were significantly more abundant in static tanks in August and May, respectively. This is in agreement with observations in many marine and freshwater systems, where static conditions favor algal groups with depth-regulating abilities, e.g., buoyancy-regulating cyanobacteria and flagellated plankton (e.g., Olli 1999). Large filamentous forms of cyanobacteria (e.g., *Anabaena, Microcystis*) contain gas vacuoles and use carbohydrate ballasting to alter buoyancy and thus allow accumulation of cells at the surface of a water column under conditions of low wind mixing (Paerl 1983; Oliver and Walsby 1984; Christian et al. 1986; Oliver 1994). The cyanobacteria sampled in the August experiment were members of the genus *Anabaena,* based upon microscope observations (Moisander personal communication). Accumulation near the surface of a turbid water column will allow cyanobacteria to compete more effectively for available light than cells that are restricted to deeper, poorly-illuminated water. Accumulation at the surface will also shade deeper phytoplankton, further increasing the competitive advantage of the cyanobacteria. Surface-dwelling cyanobacteria have been found to be extremely well adapted to the high levels of incident irradiance encountered at the surface (Eloff et al. 1976; Paerl 1983; Van Rijn et al. 1986).

In November, we found that cyanobacteria were significantly more abundant under mixed conditions. While at first this may seem contradictory, microscope observations of samples from the Neuse River show that the winter cyanobacterial populations are often composed of cells of the genera *Synechococcus* and *Synechocystis* (Paerl personal observation). These are small coccoid forms of cyanobacteria and do not have the buoyancy regulating abilities of the larger filamentous forms like *Anabaena.* These results illustrate the importance of collecting samples for microscope analysis along with samples for pigment-based assessment of phytoplankton community structure.

The success of the cryptophytes under static conditions may be linked to their ability to depth-regulate by behavioral mechanisms. The most common member of the cryptophytes, *Cryptomonas,* is a flagellated organism commonly found in samples from the Neuse River estuary (Fensin 1998). As with the cyanobacteria, the ability to depth regulate may confer a competitive advantage, allowing cells to access light near the surface of the turbid water column or to avoid supersaturating intensities (Olli 1999). In microcosm experiments with Neuse River phytoplankton, Pinckney et al. (1999) also found that static conditions favored growth of cryptophytes.

This study examined the effects of nitrogen form under irradiance conditions that closely simulated the turbid estuarine light environment (see Methods). Despite some hints that ammonium was the preferred nitrogen source in May, we found no significant differences between effects of different forms on nitrogen on phytoplankton community structure and function. This was true even though the initial composition of the phytoplankton communities used for investigation was quite different between the experiments. We hypothesized that the energetic requirements for assimilation of nitrate will preclude the use of nitrate in favor of ammonium under conditions of reduced light availability (static tanks); this generally does not seem to have been the case. We acknowledge that we were only looking at algal group-specific responses during these experiments and there may have been species-specific responses to different forms of nitrogen that were not detected by the HPLC-based technique.

The most noticeable overall response of Neuse River estuary phytoplankton communities was to mixing treatments. The effect of mixing (or lack thereof) was likely manifested directly through effects on vertical structure of the water column and indirectly through regulation of light exposure. The result was taxonomically-distinct and physiologically-distinct phytoplankton communities under mixed versus static conditions. These observations are supported by results of modeling studies that found that species composition in well-mixed waters should differ from species composition in waters of low turbulence (Huisman et al. 1999a,b). The high degree of temporal and spatial variability in phytoplankton community structure observed in the Neuse River estuary (Pinckney et al. 1997, 1998, 1999) may be primarily the result of short-term, episodic changes in the hydrodynamics of the water column that are driven by meteorological forcing rather than a result of variations in the form of nitrogen available to phytoplankton.

Results of this study have implications for management of eutrophication in the Neuse River estuary. It is already well recognized that nutrient input reductions are the only manageable option for stemming and reversing water quality degradation in this system. Presently, management action focuses primarily on reducing inputs of nitrate and ammonium to the Neuse River Basin. We have shown that when limited by nitrogen availability, phytoplankton from this system respond more or less equally to all forms of nitrogen. Management action should focus on all available nitrogen, in-

cluding organic forms, and not just nitrate and ammonium. The high degree of spatial and temporal variability in phytoplankton community structure observed in previous studies (e.g., Pinckney et al. 1998) and the marked responses that we observed with mixed versus static treatments illustrate that managing and predicting phytoplankton responses to nutrient inputs is extremely difficult. We conclude that while nitrogen availability drives accumulation of phytoplankton biomass in the Neuse River estuary, community structure seems determined more by the hydrodynamics of the system rather than by the form of available nitrogen.

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